Data Sheet

pYSG-IBA23

Cat. No.: 5-4623-001

Lot No.: 4623-

Version: 2.2
Revision Date: 04.03.2020

Description	StarGate® Acceptor Vector designed for high-level expression in yeast containing the following elements: • Copper inducible promoter (CUP1) for controlled high-level expression • URA3 auxotrophy marker for selection after transformation (do not use URA3 for selection during expression) • LEU2d auxotrophy marker for selection to increase plasmid copy number for expression (do not use LEU2d for selection after transformation) • 2µ ori for episomal replication in yeast • The expressed recombinant protein will be localized in the cytoplasm.
Yeast Expression	Cultivate transformed yeast cells under LEU2d selection until OD600 reaches 0.8 – 1.2 absorbance units. Induce protein expression by addition of copper sulphate to a final concentration of 0.5 mM.
Affinity tag	The recombinant protein will contain two affinity tags: 1. Strep-Tactin affinity tag (Strep-tagII) for the purification of recombinant protein via Strep-Tactin resins. The Strep-tagII is fused to the C-terminus of the recombinant protein. 2. GST-tag (Glutathione-S-Transferase) for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein. After purification the GST may be removed by digesting with PreScission™ Protease.
Resistance	Ampicillin
Form	5 µg, dissolved in 20 µl TE buffer, pH 8,0: 10 mM Tris-HCl, 1 mM EDTA
Concentration	250 ng/µl
Stability	12 months after shipping
Storage	recommended: 2-8 °C for frequent usage, -20 °C for long-term storage
Shipping	room temperature
Hazards	Product is not classified as hazardous according to (EC) No 1272/2008 [CLP]. A Material Safety Data Sheet is provided.

Note: The sequences have been compiled from information in the sequence database, published literature, and other sources, together with partial sequences obtained by IBA, however, the vectors have not been completely sequenced.
PreScission™ Protease is a trademark of GE HEALTHCARE
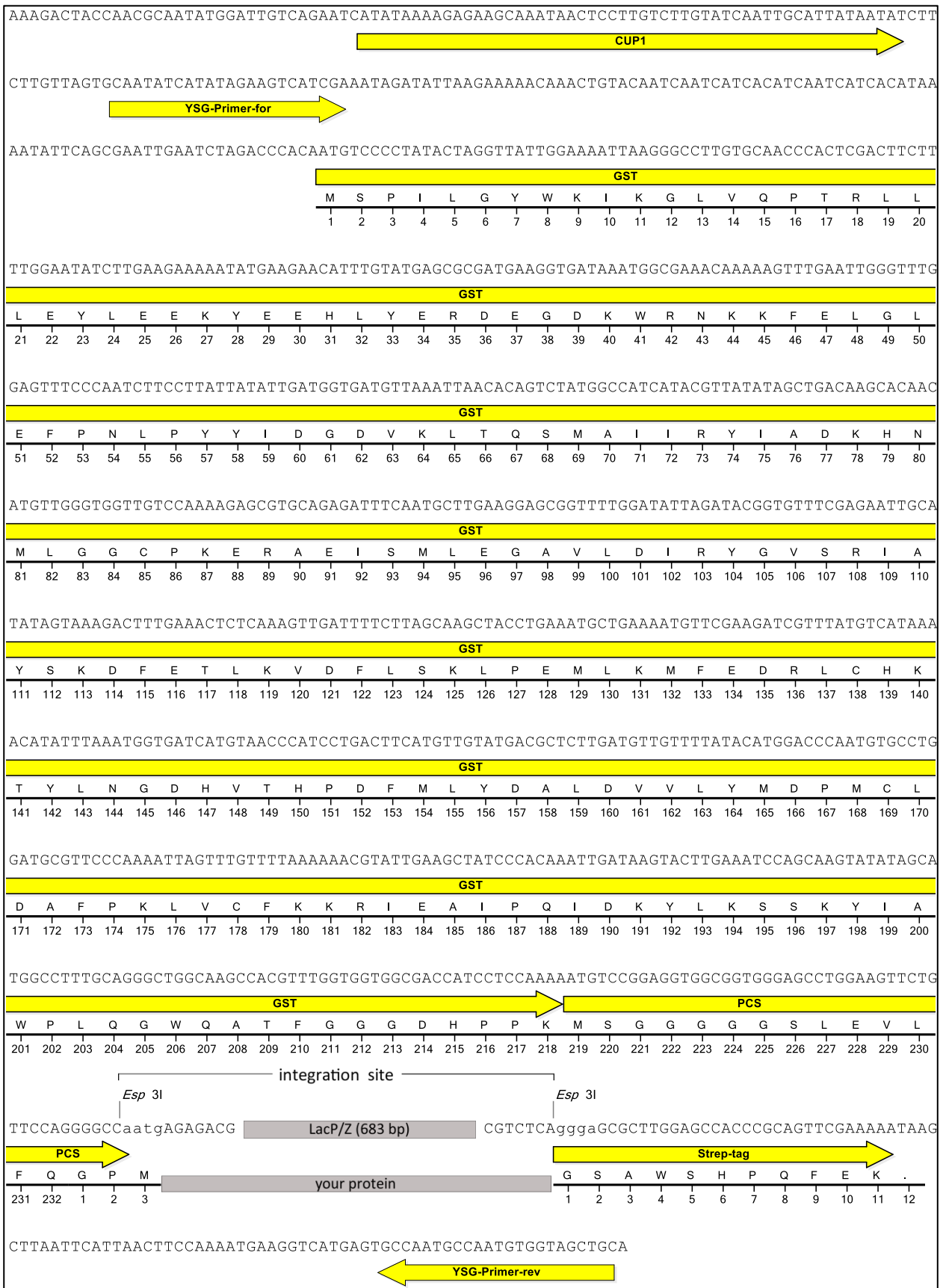
For research use only

Expression cassette of pYSG-IBA23

```
AAAGACTACCAACGCAATATGGATTGTCAGAATCATATAAAAGAGAAGCAAATAACTCCTTGTCTTGTATCAATTGCATTATAATATCTT
```
CUP1 →

```
CTTGTTAGTGCAATATCATATAGAAGTCATCGAAATAGATATTAAGAAAAACAAACTGTACAATCAATCATCACATCAATCATCACATAA
```
← YSG-Primer-for →

```
AATATTCAGCGAATTGAATCTAGACCCACAATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTT
```

GST																			
M	S	P	I	L	G	Y	W	K	I	K	G	L	V	Q	P	T	R	L	L
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

```
TTGGAATATCTTGAAGAAAAATATGAAGAACATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
```

GST																			
L	E	Y	L	E	E	K	Y	E	E	H	L	Y	E	R	D	E	G	D	K	W	R	N	K	K	F	E	L	G	L
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50

```
GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC
```

GST																			
E	F	P	N	L	P	Y	Y	Y	I	D	G	D	V	K	L	T	Q	S	M	A	I	I	R	Y	I	A	D	K	H	N
51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80

```
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCA
```

GST																			
M	L	G	G	C	P	K	E	R	A	E	I	S	M	L	E	G	A	V	L	D	I	R	Y	G	V	S	R	I	A
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110

```
TATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAA
```

GST																			
Y	S	K	D	F	E	T	L	K	V	D	F	L	S	K	L	P	E	M	L	K	M	F	E	D	R	L	C	H	K
111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140

```
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCTG
```

GST																			
T	Y	L	N	G	D	H	V	T	H	P	D	F	M	L	Y	D	A	L	D	V	V	L	Y	M	D	P	M	C	L
141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170

```
GATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
```

GST																			
D	A	F	P	K	L	V	C	F	K	K	R	I	E	A	I	P	Q	I	D	K	Y	L	K	S	S	K	Y	I	A
171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200

```
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAAATGTCCGGAGGTGGCGGTGGGAGCCTGGAAGTTCTG
```

GST																		PCS			
W	P	L	Q	G	W	Q	A	T	F	G	G	G	D	H	P	P	K	M	S	G	G	G	G	S	L	E	V	L
201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230

integration site —————

Esp 3I   *Esp* 3I

```
TTCCAGGGGCCaatgAGAGACG   [ LacP/Z (683 bp) ]   CGTCTCAgggaGCGCTTGGAGCCACCCGCAGTTCGAAAAATAAG
```
← PCS →   Strep-tag →

F	Q	G	P	M		[your protein]		G	S	A	W	S	H	P	Q	F	E	K	.
231	232	1	2	3				1	2	3	4	5	6	7	8	9	10	11	12

```
CTTAATTCATTAACTTCCAAAATGAAGGTCATGAGTGCCAATGCCAATGTGGTAGCTGCA
```
← YSG-Primer-rev
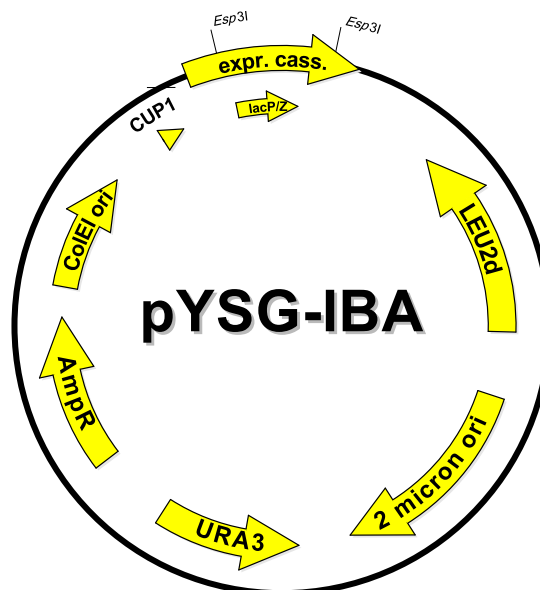
Expression cassette of pYSG-IBA23, continued

LacP/Z cassette = contains LacZ alpha fragment under control of a separate promoter, which allows alpha complementation of *LacZ* mutations such as *LacZΔM15* as in *E. coli* DH5α or TOP10.

your protein = after StarGate cloning using *Esp3*I your gene of interest will be located here

Features	from bp	to bp		Sequencing primer
LEU2d	1668	574		**YSG-Primer-for (Cat. No. 5-0000-141)**
2 micron ori	2032	3194		
URA3	4293	3490		5'– CAATATCATATAGAAGTCATCGA –3'
Ampicillin resistance gene	4725	5585		
ColEIori	5756	6345		
CUP1 promoter	6873	6925		**YSG-Primer-rev (Cat. No. 5-0000-142)**
forward primer binding site	6939	6961		5'– GCAGCTACCACATTGGCATTGGC –3'
GST-tag	7049	7702		
PreScission™ Protease site (PCS)	7703	7750		
LacZ alpha fragment	7979	8380		
Strep-tag	8444	8476		
reverse primer binding site	8517	8539		
total vector length		8540		

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